



Evaluation of Cassava Varieties for Tolerance to Water Deficit Stress conditions

Roots and tubers have so far been regarded as inferior and neglected food crops even in areas where they are staples (Horton, 1988). Cassava (*Manihot esculenta* Crantz.) is one of those major tropical tuber crops grown across the tropical regions of the world starting from Southern America to South East Asia and Australia through the Sub-Saharan Africa. It is an important source of energy for the millions of people in the tropical and subtropical parts of the world (Yan et al. 2013). It has spread to the Central America in the 11th century through human migrations and to India in the 17th century by Portuguese people. Cassava is largely cultivated for human consumption in Kerala and for starch in Tamil Nadu. Besides, it is also grown in Andhra-Pradesh, Assam, Karnataka, Madhya-Pradesh, Pondicherry, Nagaland, Tripura, Mizoram and the Andaman-Nicobar islands. It produces more calories per unit area per unit time than any other crop. India claims the highest productivity of cassava (27.9 t ha⁻¹) in the world (George et al. 2011). Cassava is mainly grown for its starchy tubers of edible and commercial value. It is an important source of starch and a component of animal, fish and poultry feeds (Abraham et al. 2006; George et al. 2011). Moreover, it is also used in various industries including starch and starch-derived products such as sago, textile, alcohol and high fructose-glucose syrups (Joseph et al. 2004; Yan et al. 2013). In cassava tubers, starch is present in pure form as it is free from proteins and lipids. Cassava starch is also being used in the manufacture of biodegradable plastic and processed products like baby food, vermicelli, chips and papads (Abraham et al. 2006).

Climate change is an undeniable truth and its consequences are being experienced by us. Drought is one of the most important outgrowths of erratic rainfall and shift in monsoon patterns. Kerala is one of the states receiving a high amount of rainfall in the country. In fact, Southwest monsoons enter the country through Kerala. Unfortunately, under changing climatic situations, Kerala is facing drought since past few years

(IMD 2017). All the districts in Kerala received a low amount of rainfall during Southwest monsoons ranging from 24% deficit in Ernakulam to 59% deficit in Wayanad in 2016 (IMD 2017).

Cassava is said to be drought tolerant crop, compared to other vegetable and more water requiring crops. The drought tolerance in cassava has not been quantified and it appears to be more related to survival (Daryanto et al. 2016). The critical period of irrigation in cassava is initial 3-4 months after planting (George et al. 2001; Laban et al. 2013). Water Deficit Stress (WDS), during the early period of establishment, causes considerable damage to the plants. In Uganda, 84.27% mortality due to early period of WDS was observed within three months after planting (Laban et al. 2013). The delay in planting due to the delay in monsoon followed by drought significantly reduce the crop yield up to 30% further affecting root bulking (George et al., 2001). For several decades, studies have examined the problems and potentials of root/tuber crops production, but limited progress has been made in improving the productivity of most of these crops under drought conditions (Daryanto et al. 2016). With this background, an experiment was conducted to study the WDS tolerance of cassava varieties under WDS conditions during 2 months after planting (MAP) to 5 MAP.

Early period drought tolerance was studied in a poly house in the block-I of ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala with 15 varieties (M-4, H-97, H-165, H-226, Sree Jaya, Sree Vijaya, Sree Padmanabha, Vellayani Hraswa, Sree Visakhm, Sree Sahya, Kalpaka, Sree Prakash, Sree Swarna, Sree Reksha and PDPCMR-1) with two replications under two treatments *viz.*, irrigated and WDS under potted conditions. For plants under the WDS conditions irrigation was withdrawn at two months after planting for a period of 10 days at every month up to 5 months after planting. During the remaining 20 days, the plants were watered at 2 days intervals. The plants under irrigated conditions were irrigated at every alternate day. Observations on plant height, number of

leaves per plant, fresh leaf weight, fresh stem weight, leaf total sugars and leaf HCN content were recorded among the 15 varieties under both the treatments.

Total sugar content was measured by using anthrone reagent as per the method of Sadasivam and Manickam (1991). A dried sample of 0.1 g weight was taken and the total sugars were extracted with 5 ml of 2.5N HCl at 60°C for three hours on a water bath. The extract was filtered through filter paper and the final volume was made up to 10 ml. Anthrone reagent was prepared by dissolving the 200 mg of anthrone in 100 ml of concentrated sulphuric acid. Two ml of extract was mixed with 4 ml of ice cold anthrone and was kept in a water bath at 100°C for 10 minutes. Immediately it was cooled and absorbance was measured at 620 nm. The standard curve was prepared by using various concentrations of glucose (20, 40, 60, 80 and 100 µg ml⁻¹). The total sugar content in the sample was calculated by using the standard curve and expressed in glucose equivalents.

The HCN content in the leaves was measured through alkaline picrate method. Alkaline picrate was prepared by dissolving the 5g picric acid and 20g Na₂CO₃ in 1 L water. Filter paper was cut into pieces of 20 square centimeter size and immersed in the above solution. One gram of dried leaf sample was transferred after crushing with 25ml of H₂O into a 500ml conical flask which was covered with a cork onto which the strip of filter paper was attached. The same was kept for overnight at room temperature. Next day the filter paper strip was collected and cut into small pieces and put into 60ml H₂O. The absorbance was measured at 515nm. The HCN concentration was calculated by using the standard graph.

Paired t-test values confirmed that there was significant difference between control and WDS plants for plant height, number of leaves per plant (Table 1), fresh leaf and stem weight per plant, total fresh biomass per plant (Table 2), total carbohydrate content of leaf and stem and leaf HCN content (Table 3). The mean of 15 cassava varieties of all the above traits were found reduced under the WDS conditions than the irrigated conditions.

Before inducing the WDS there was no significant difference for plant height between plants under irrigated (43.9± 3.29 cm) and WDS conditions (37.3± 1.73 cm). But, after inducing the WDS there were significant differences between the two treatments. The difference in the mean plant height of 15 cassava varieties at 7 days after inducing the WDS conditions during the 3rd month was 34.63± 4.71 cm and the difference increased to 82.1± 11.7 cm at 7 days after inducing the WDS during in the 5th month (Table 1). In the present study, at 5 MAP after inducing the WDS three times between 3rd and 5th month, a reduction of 47.4± 4.5% was observed in mean plant height. The variety Sree Jaya suffered the maximum reduction in pant height (71.2%) followed by Sree Sahya (68.9%) and PDPCMR-1 (66.9%) while the varieties H-226 (14.4%), Kalpaka (26.1%) followed by Sree Reksha (31.2%) and Vellayani Hraswa (33%) had the lowest reduction in plant height under WDS conditions relative to irrigated plants. The variety Sree Visakhm had the maximum plant height (125 cm) while Sree Sahya had the minimum plant height (47 cm) under WDS conditions (Table 4).

Likewise, there was no significant difference for number of leaves between plants under irrigated (19.80± 0.63) and plants under WDS conditions (18.03± 1.19 cm) before inducing the WDS. But, a difference in number of leaves was found between

Table 1. Mean of plant height and number of leaves of 15 cassava varieties as affected by WDS conditions

	Plant height (cm)						Number of leaves per plant		
	Before inducing the stress	7 days after 1 st stress	7 days after 2 nd stress	7 days after 3 rd stress	Before inducing the stress	7 days after 1 st stress	7 days after 2 nd stress	7 days after 3 rd stress	
Irrigated	43.9± 3.29	78.19± 4.55	125.27± 8.18	162.4± 12.5	19.80± 0.63	24.2± 0.68	30.7± 2.18	30.7± 2.18	
WDS	37.3± 1.73	43.17± 2.28	56.01± 2.97	80.3± 5.9	18.03± 1.19	10.8± 0.94	11.3± 0.80	11.3± 0.80	
T cal value	1.73	7.36*	8.96*	7.01*	1.41	7.56*	7.10*	7.10*	

*Significant at 5% level

Table 2. Mean of fresh leaf weight, fresh stem weight and total fresh biomass per plant as affected by WDS conditions

	Fresh leaf weight (g plant ⁻¹)	Fresh stem weight (g plant ⁻¹)	Total fresh biomass (g plant ⁻¹)
Irrigated	61.83± 9.99	144.5± 63.8	206.3± 14.0
WDS	9.17± 1.55	24.6± 1.8	33.8± 2.3
T cal value	5.16*	7.14*	12.54*

*Significant at 5% level

Table 3. Mean of total carbohydrate content of leaf, stem, leaf HCN content as affected by WDS conditions

	Total carbohydrate in dry leaf (g100g ⁻¹)	Total carbohydrate in dry stem (g100g ⁻¹)	Dry leaf HCN (ppm)
Irrigated	4.073± 0.186	2.828± 0.28	181.7± 19.5
Moisture stress	1.381± 0.060	1.932± 0.26	52.3± 9.8
T cal value	15.01*	4.45*	6.29*

*Significant at 5% level

plants under irrigated 30.7± 2.78 and WDS 11.3± 0.80 conditions at 7 days after inducing the WDS during the 4th month. But, a difference in number of leaves was found between months under irrigated (30.7± 2.78) and WDS (11.3± 0.80) condition (Table 1).

The mean fresh leaf weight stem weight and total biomass of 15 cassava varieties was significantly reduced to 52.7± 10.2, 119.9± 16.8 and 172.6± 13.8 g respectively in plants at 5 MAP under the WDS conditions relative to the irrigated plants (Table 2). At 5 MAP after inducing the WDS three times between 3rd and 5th month, a reduction of 80.7± 3.3% in mean leaf weight was observed in plants under WDS conditions relative to irrigated plants. The varieties PDPCMR-1 had the maximum fresh leaf weight (27.16 g) while Sree Visakhm and Sree Prakash had the minimum fresh leaf weight (5.03 g). The leaf weight was reduced in the variety Sree Visakhm by 97.1%, in Sree Rekasha by 96% and in Sree Prakash by 93.3% while Sree Padmanabha (59.8%) had the minimum reduction in leaf weight (Table 4).

At 5 MAP after inducing the WDS three times between 3rd and 5th month, a reduction of 79.5± 3.2% in mean stem weight was observed in plants under WDS conditions relative to irrigated plants. A very high reduction of 92.2% in stem weight was recorded in the variety Sree Jaya followed by Sree Padmanabha (91.6%) and M-4 (90.4%) under WDS conditions relative to irrigated plants, while the variety Sree Visakhm (45.9%) had the minimum reduction in the stem weight (Table 4).

At 5 MAP after inducing the WDS three times between 3rd and 5th month, a reduction of 82.9± 1.5% in mean total fresh biomass was observed in plants under WDS conditions relative to irrigated plants. The variety Sree Jaya suffered the maximum reduction (91.6%) of total fresh biomass followed by the variety Sree Prakash (88.5%) whereas the variety Vellayani Hraswa (71%) had the minimum reduction in total biomass under WDS conditions relative to control conditions (Table 4). Tuber formation was not observed under WDS conditions while small tubers were present under irrigated conditions.

Helal et al. (2013) also found that WDS reduced the plant height, stem diameter, leaf area, fresh and dry weight of shoots and roots, root length and diameter of cassava compared to unstressed control plants. The findings in the present experiment corroborated with these findings. Laban et al. (2013) indicated a reduction of 25.72% in leaf retention and Duque and Setter (2013) displayed a reduction in total sugar content in leaves in cassava under WDS conditions. A reduction of 32.95% in plant height, 54.95% in shoot yield and 26.15% in tuber dry weight in cassava under drought conditions was previously reported by Oliveira et al (2017). Ravi and Saravanan (2001a) found high foliage weight in Sree Visakhm when compared with M-4, H-165, H-226 and CI-21 under WDS conditions. The reduction in the leaf area under WDS in cassava as it occurs to reduce the transpirational water loss (Alves and Setter 2004).

Table 4. The percentage of mean plant height, fresh leaf weight, fresh stem weight and total fresh biomass per plant at five months after planting

Varieties	Plant height (cm)		Fresh leaf weight (g plant ⁻¹)		Fresh stem weight (g plant ⁻¹)		Total fresh biomass (g plant ⁻¹)			
	Irrigated	WDS	Reduction (%)	Irrigated	WDS	Reduction (%)	Irrigated	WDS		
M-4	174.0	97.5	44.0	40.0	13.08	67.3	330.0	370.0	44.6	87.9
H-97	201.5	94.5	53.1	25.0	7.04	71.8	125.0	150.0	33.9	77.4
H-165	185.5	66.0	64.4	65.0	7.54	88.4	90.0	155.0	26.2	83.1
H-226	80.0	68.5	14.4	37.5	12.07	67.8	150.0	187.5	27.8	85.2
Sree Jaya	212.0	61.0	71.2	35.0	4.02	88.5	180.0	215.0	18.1	91.6
Sree Vijaya	146.0	98.0	32.9	45.0	4.02	91.1	140.0	185.0	38.1	79.4
Sree Padmanabha	155.5	59.0	62.1	25.0	10.06	59.8	215.0	240.0	28.2	88.3
Sree Visakhham	201.5	125.0	38.0	175.0	5.03	97.1	65.0	240.0	40.2	83.3
Sree Sahya	151.0	47.0	68.9	70.0	7.34	89.5	125.0	195.0	27.3	86.0
Kalpaka	94.0	69.5	26.1	50.0	6.04	87.9	135.0	185.0	27.3	85.3
Sree Prakash	119.0	55.3	53.6	75.0	5.03	93.3	160.0	235.0	27.1	88.5
Sree Swarna	166.0	79.5	52.1	60.0	12.07	79.9	147.5	207.5	36.1	82.6
Sree Reksha	170.0	117.0	31.2	100.0	4.02	96.0	85.0	185.0	37.1	79.9
PDPCMR-1	265.0	89.5	66.2	90.0	27.16	69.8	110.0	200.0	52.6	73.7
Vellayani Hraswa	115.0	77.0	33.0	35.0	13.08	62.6	110.0	145.0	42.1	71.0
Mean	162.4	80.3	47.4	61.8	9.17	80.7	144.5	206.3	33.8	82.9
S.E	12.5	5.90	4.50	10.0	1.55	3.30	16.50	14.0	2.30	1.50

The mean total leaf sugar content in leaves of 15 cassava varieties was found to be reduced to $1.381 \pm 0.06 \text{ g } 100\text{g}^{-1}$ on dry weight basis (dw) in plants under WDS conditions as compared to irrigated plants ($4.073 \pm 0.186 \text{ g } 100\text{g}^{-1}$ dw). The varieties Sree Sahya (75.8%) had the maximum reduction in total leaf sugar content followed by Sree Prakash (73.3%), Sree Reksha (72.7%) and H-97 (72.4%) under WDS conditions. The minimum reduction in total leaf sugar content was noted in the variety Sree Swarna (50.1%). Sree Padmanabha ($1.78 \text{ g } 100\text{g}^{-1}$ dw) followed by Sree Visakhham ($1.71 \text{ g } 100\text{g}^{-1}$ dw) had the maximum leaf total sugar content in the leaf under WDS conditions. The variety PDPCMR-1 had the minimum total leaf sugar content ($0.977 \text{ g } 100\text{g}^{-1}$) under WDS conditions relative to irrigated conditions (Table 5).

A reduction in total sugar content in the stem was observed in all the varieties under WDS conditions plants relative to irrigated conditions. The mean of total carbohydrate content of the stem in 15 cassava varieties was reduced to $2.828 \pm 0.28 \text{ g } 100\text{g}^{-1}$ dw in plants under WDS relative to plants under irrigated conditions ($1.932 \pm 0.26 \text{ g } 100\text{g}^{-1}$ dw). The minimum reduction of 6.0% was observed in the stem of variety Sree Jaya followed by Kalpaka (9.60%). The maximum reduction of 66.1% was observed in the stem of variety H-165 followed by Vellayani Hraswa (62.7%). The variety Kalpaka had the maximum total carbohydrate content in the stem ($4.753 \text{ g } 100\text{g}^{-1}$) followed by Sree Jaya ($2.880 \text{ g } 100\text{g}^{-1}$) under WDS conditions relative to plants under irrigated conditions (Table 5).

The mean leaf HCN content in 15 cassava varieties was reduced to 183.8 ± 18.5 ppm in plants under WDS conditions than irrigated plants (47.2 ± 9.3 ppm). Maximum reduction in the HCN content was found in the leaves of variety Sree Reksha (97%) followed by Kalpaka (94.6%) while the minimum reduction was found in the leaves of variety Sree Jaya (22.6%) followed by H-226 (45.3%) under WDS condition relative to plants under irrigated conditions (Table 5).

A reduction in the rate of photosynthesis in cassava was observed under WDS conditions by Ravi and

Table 5: Mean of total sugar content of leaf, stem, leaf HCN content at five months after planting

Varieties	Total sugars in dry leaf (g 100g ⁻¹)			Total sugars in dry stem (g 100g ⁻¹)			Dry leaf HCN (ppm)		
	Control	Stress	Reduction(%)	Control	Stress	Reduction(%)	Control	Stress	Reduction(%)
M-4	3.192	1.265	60.4	3.004	2.576	14.2	185.5	24.9	86.6
H-97	4.691	1.296	72.4	2.965	2.469	16.7	176.5	24.7	86.0
H-165	4.337	1.468	66.2	3.391	1.130	66.7	279.7	53.5	80.9
H-226	3.070	1.210	60.6	2.019	1.004	50.3	223.0	121.9	45.3
Sree Jaya	3.845	1.273	66.9	3.065	2.880	6.00	147.2	113.9	22.6
Sree Vijaya	4.204	1.585	62.3	1.798	1.015	43.5	183.6	46.5	74.7
Sree Padmanabha	5.156	1.782	65.4	3.081	2.172	29.5	81.7	24.9	69.5
Sree Visakhham	4.343	1.708	60.7	2.229	1.533	31.2	148.5	56.2	62.2
Sree Sahya	5.112	1.235	75.8	1.643	1.087	33.8	307.3	89.4	70.9
Kalpaka	3.983	1.194	70.0	5.255	4.753	9.60	127.9	6.9	94.6
Sree Prakash	4.857	1.296	73.3	3.341	2.209	33.9	168.4	24.9	85.2
Sree Swarna	3.358	1.674	50.1	2.030	1.750	13.8	306.7	48.9	84.1
Sree Reksha	4.437	1.211	72.7	2.014	1.237	38.6	169.4	5.1	97.0
PDPCMR-1	3.081	0.977	68.3	1.704	1.351	20.7	63.6	19.5	69.3
Vellayani Hiraswa	3.435	1.548	54.9	4.879	1.820	62.7	188.1	46.0	75.5
Mean	4.073	1.381	65.3	2.828	1.932	31.4	183.8	47.2	73.6
S.E	0.186	0.060	1.90	0.280	0.260	4.81	18.5	9.30	5.00

Saravanan (2001b). This might be the reason for the reduction in the net photosynthates (carbohydrates) in the leaf and stem under WDS conditions. Previously, Duque and Setter (2013) also reported that in leaf blades and petioles, sugars were depleted rapidly during WDS. They further mentioned that stems gradually lost starch and had sufficient reserves to serve as a prolonged source of remobilized carbohydrate during WDS. Tan (1995) reported an increase in the HCN content in the roots under WDS conditions. In the present experiment, a decrease in HCN content in the leaves was observed under WDS conditions when estimated on dry matter basis. The acetone cyanohydrin which is formed from the breakdown of the linamarin in the presence of linamarase reacts with the glucose and forms acetone and HCN (Siritunga and Sayre, 2007). Hence, the low HCN content in leaves under WDS condition could possibly be explained as the low carbohydrate content in the leaves under WDS conditions might led to the limited availability of glucose to acetone cyanohydrin.

It can be concluded that water deficit stress conditions diminish the plant growth and development in cassava as witnessed by the reduction in the plant height, number of leaves, fresh leaf weight, fresh stem weight and total fresh biomass. The variety, Sree Jaya was found to be highly susceptible to WDS conditions after 2 MAP as it had the maximum reduction of these parameters under WDS conditions. All the varieties under study had experienced 71 to 91.6% loss in total fresh biomass under WDS conditions. The varieties, Sree Padmanabha, Sree Visakhham and Sree Swarna maintained high leaf total sugars content under WDS conditions and the variety, Kalpaka had the maximum total sugar content in the stem. Hence, these varieties may be used for improving WDS tolerance for cassava.

Acknowledgement

The authors are thankful to Dr. T. Makesh kumar for extending the polyhouse facility, Dr. G. Padmaja and Mrs. Mitra for helping in the standardization and estimation of total sugars

content and to Dr. K. Susan John for sharing the protocol and facility for the estimation of HCN content.

References

- Abraham, K., Edison, S., Unnikrishnan, M., Sheela, M. N., Vimala, B. Sreemukari, M. T. and Naskar, S. K. 2006. Tuber Crop Varieties released by the Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala, India.
- Alves, A.A.C. and Setter, T.L. 2004. Abscisic acid accumulation and osmotic adjustment in cassava under water deficit. *Envi. Exp. Bot.*, **51**: 259-271.
- Daryanto, S., Wang, L. and Jacinthe, P. A. 2016. Drought effects on root and tuber production: A meta-analysis. *Agri. Water Manage.*, **176**: 122-131.
- Duque, L. O. and Setter, T. L. 2013. Cassava response to water deficit in deep pots: root and shoot growth, ABA, and carbohydrate reserves in stems, leaves and storage roots. *Tropical Pl. Biol.*, **6**: 199-209.
- George, J., Mohankumar, C. R., Nair, G. M. and Ravindran, C. S. 2001. Cassava agronomy research and adoption of improved practices in India: Major achievements during the past 30 years. Proceedings of the 6th Regional Workshop on Cassava's Potential in Asia in the 21st Century: Present Situation and Future Research and Development Needs, Ho Chi Minh City, Vietnam, 279-299.
- George, J., Suresh Kumar, P. and Unnikrishnan, M. 2011. Description of recommended/ released varieties under AICRP on tuber crops, Technical bulletin series No. 51. All India Coordinated Research Project on Tuber Crops, ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram.
- Helal, N. A. S., Eisa, S. S. and Attia, A. 2013. Morphological and chemical studies on influence of water deficit on cassava. *World J. Agri. Sci.*, **9**(5): 369-376.
- Horton, D. E. 1988. Underground crops: long-term trends in production of roots and tubers. Winrock International Institute for Agricultural Development, Morrilton, AR, USA.
- IMD, 2017. Season's Rainfall 2017, Meteorological Centre, Thiruvananthapuram, India Meteorological Department. http://imdtvm.gov.in/index.php?option=com_content&task=view&id=23&Itemid=37
- Joseph, R., Yeoh, H. H. and Loh, C. S. 2004. Induced mutations in cassava using somatic embryos and the identification of mutant plants with altered starch yield and composition. *Pl. Cell Rep.*, **23**: 91-98.
- Laban, T. F., Kizito, E. B., Baguma, Y. and Osiru, D. 2013. Evaluation of Ugandan cassava germplasm for drought tolerance. *Inter. J. Agri. Crop Sci.*, **5**(3): 212-226.
- Oliveira, E. J., Morgante, C. V., Chaves, A. R. M., Cruz, J. L., Aidar, S. T., Antonio, R. P., Filho, M. A. C. 2017. Evaluation of cassava germplasm for drought tolerance under field conditions. *Euphytica*, 213:188.
- Ravi, V. and Saravanan, R. 2001a. Photosynthesis and productivity of cassava under water deficit stress and stress free conditions. *J. Root Crops*, **27**(1): 214-218.
- Ravi, V. and Saravanan, R. 2001b. Characteristics of photosynthesis and respiration in cassava and sweet potato. *J. Root Crops*, **27**(1): 258-262.
- Sadasivam, S. and Manickam A. 1991. Biochemical methods. New Age International Publishers.
- Siritunga, D. and Sayre, R. T. 2007. Transgenic approaches for cyanogen reduction in cassava. *J. AOAC Inter.*, **90**: 1450-1455.
- Tan, S. L. 1995. Factors affecting cyanide content in cassava (*Manihot esculenta* Crantz) *MARDI Res. J.*, **23**(2): 121-131.
- Yan, H., Lu, L., Hershey, C., Ceballos, H., Chen, S. and Li, K. 2013. Cassava mutation breeding: current status and trends. *Pl. Mutation Rep.*, **3**(1): 37-44.

Division of Crop Improvement, ICAR- Central Tuber Crops Research Institute, Sreekariyam,

Corresponding Author: A. V. V. Koundinya;
email: koundi.hortico@gmail.com

A. V. V. Koundinya
Vivek Hegde
M. N. Sheela
C. Visalakshi Chandra